Applicants note that the Examiner has acknowledged Application's election with traverse of Group I in the response to restriction requirement filed May 22, 2001. The Examiner states that claims 1-11, 14 and 15 are under consideration. The objections and rejections set forth in the Office Action are addressed below in the order in which they are raised.

Priority

The Office Action states that, should Applicants desire the benefit of priority to their provisional application U.S. 60/103,787, specific reference to the earlier filed application must be made in the instant application. Accordingly, the specification has been amended above so as to insert a claim to the benefit of U.S. 60/103,787, and an incorporation by reference of the contents of this provisional application, as the first sentence of the application following the title.

Specification

The specification has been objected to as containing certain remaining nucleotide and/or amino acid sequences disclosures, encompassed by definitions set forth in 37 CFR 1.821(a)(1) and (a)(2), that are not yet in compliance with 37 CFR 1.821(d) which requires the use of assigned sequence identifiers in all instances. Accordingly, a sequence identifier for each such sequence disclosure has been inserted by amendment- as directed above. These amendments add no new matter.

REJECTIONS

Rejections Under 35 U.S.C. §112, first paragraph-Written Description

The Office Action states that claims 1-11, 14 and 15 have been rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant are that the inventor(s), at the time the application was filed, had possession of the claimed invention. In particular, the Office Action states that, while the specification teaches a method of use based on the "use of Cdc4 and its human analog, βTrCP, for degradation of a polypeptide," "the scope of the claims includes numerous structural variants" (and that) "the specification fails to describe any other representative species by any identifying characteristics or properties other than the functionality

of being (a) ubiquitin ligase" (and further that) "specific guidance is needed on the interaction, if it exists, of various members of the ubiquitin ligase family with various E2 ubiquitin conjugating enzymes and F-box binding proteins." Applicants respectfully traverse this rejection for the reasons which follow.

The written description requirement is satisfied where the skilled artisan, reading the specification, would be satisfied that the patent applicant was in possession of the claimed invention at the time of filing. See *In re Edwards*, 568 F.2d 1349 (C.C.P.A. 1978). Notably, the written description requirement is not to be confused with a requirement for literal support, although this question does not appear to be in issue, as the challenged claims were presented in the application as filed. Rather the rejection appears to challenge the scope of the claims based upon the recited examples without consideration of the totality of teachings of the specification.

In particular, the application teaches the surprising and useful observation that a selected polypeptide (such as the retinoblastoma RB protein) ~~can be targeted for degradation~~ *in vivo* by simply expressing a fusion protein which recruits the selected target protein, via protein/protein interactions, to a ubiquitin ligase. The application teaches that a key aspect to achieving success in recruiting the target polypeptide to a ubiquitin ligase is the creation of a fusion protein, an elemental aspect of genetic engineering which is well-known and, indeed, routine in the art. The application further teaches the critical aspects of the fusion protein- namely that it be composed of a ubiquitin protein ligase polypeptide and a target polypeptide interaction domain. Specific ubiquitin protein ligases are described in detail in the application- particularly the HECT ubiquitin ligases, the Ubr1p ubiquitin ligases and the SCF ubiquitin ligases- which include F-box ubiquitin ligase polypeptide components. Indeed numerous exemplary suitable SCF ubiquitin ligase polypeptides, specifically F-box polypeptides, are described in detail in the application (see pages 146-177). Moreover, the skilled artisan has access to numerous other F-box polypeptides - e.g. by searching for sequences which are described as containing an F-box motif or by searching for homology to the described F-box protein. In addition, the HECT ubiquitin ligases are well known in the art and can readily be identified and isolated by the skilled artisan using these approaches - see e.g. the following HECT ubiquitin ligases: human NEDD-4 ubiquitin ligase protein (see GenBank Accession No. P46934); mouse NEDD-4 ubiquitin ligase protein (see GenBank Accession No. P46935); and yeast RSP5 ubiquitin ligase protein (see

GenBank P39940). Still further, the N-end rule ubiquitin ligases were described in the application and known and available to the skilled artisan at the time of the invention- see e.g. the UBR1 family of N-end rule ubiquitin ligase polypeptides including: human hyd ubiquitin ligase corresponding to GenBank Accession No. AAP88143; the mouse ubiquitin protein ligase corresponding to GenBank Accession No. AAC23678; and the *S. pombe* ubiquitin ligase corresponding to GenBank Accession No. O13731. Accordingly, the teachings of the application are sufficient to inform the person of ordinary skill in the art that the applicants were in possession of the "ubiquitin protein ligases" of the invention.

Nevertheless, in order to expedite prosecution, and not in acquiescence to the Examiner's rejection, Applicants have amended the relevant claims so as to incorporate the ubiquitin ligase polypeptide classes described in the application (see amended claims 1, 6 and 11 and canceled claim 5). Applicants expressly reserve the right to pursue without prejudice the originally filed claims as well as other disclosed subject matter at a latter date. As a result of the amendment, the claimed invention includes a set of ubiquitin ligase classes which each possess certain well-known structural features (e.g. an "F-box", a "HECT" domain or a UBR1-homologous domain). Accordingly, the invention is not claimed as "a genus of molecules described (merely) by function" as asserted on page 4 of the Office Action. In summary, Applicants believe that the rejection under 35 U.S.C. § 112, 1st paragraph (written description) is in error, but that, notwithstanding the reservation of a right to pursue the originally filed claims, the proposed amendments obviate this rejection in any event. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection in light of the proposed claim amendments.

Rejections Under 35 U.S.C. §112, first paragraph-Enablement

The Office Action further states that claims 1-11, 14 and 15 have been rejected under 35 U.S.C. § 112, 1st paragraph (enablement) "because the specification, while being enabling for a method of use of Cdc4 and bTrCP hybrids for degradation of polypeptides in yeast and human cells, respectively, does not reasonably provide enablement for a method of use of any hybrid based on any component of ubiquitin ligase...". Applicants respectfully traverse this rejection for the reasons which follow.

First, Applicants believe that the proposed amendments described above, made with the express reservation of rights to pursue without prejudice the originally filed claims as well as other disclosed subject matter at a latter date and without acquiescence to either the written description or the instant enablement rejection, in any case obviate the rejection for lack of enablement. Notwithstanding this, the remaining relevant specific concerns expressed in the Office Action are addressed below.

The Office Action states that the claimed invention "amounts to any hybrid polypeptide comprising peptide structures both naturally-occurring and man-made." To the extent that this asserts that the claimed invention reads on naturally-occurring ubiquitin ligase polypeptides which possess inherent target recruitment domains (such as the WD40 motif or the leucine rich repeats of the F-box proteins- see page 5, lines 1-2), Applicants respectfully disagree. Applicants submit that the claimed invention is specific to the use of a "ubiquitin protein ligase polypeptide-target polypeptide interaction domain fusion protein" in which the target polypeptide interaction domain is not originally present in the ubiquitin protein ligase but is introduced by genetic engineering and is essential to the functioning of the system. Accordingly, the skilled artisan would understand that the claimed invention does not read on the use of native (non-fusion) ubiquitin ligase polypeptides for recruitment of target polypeptides.

The Office Action further states that the term "ubiquitin protein ligase" encompasses diverse proteins. The above-described amendments obviate this aspect of the rejection.

The Office Action states that "Cdc4 is auto-ubiquitinated by Cdc34p/SCF^{Cdc4p} complex while other components of this complex, Skp1 and Cdc53....are not (specification, page 127)". Applicants note that, while the auto-ubiquitination of Cdc4 and other F-box protein provides for a particularly interesting "self-limiting" character to this embodiment of the invention, the teachings of the application provide broad support for the use of any of the claimed ubiquitin ligase polypeptide components. This is so because, as taught in the application, the invention is based upon recruitment *per se* of the target polypeptide to the ubiquitin ligase. This point also applies with equal force to the assertions on page 8 of the Office Action which regard claim 11 specifically.

The Office Action further states that it is "unknown whether the method can be used in a bacterial cell", however the specification clearly describes (see e.g. page 14, lines 5-6 and 14--17), and the skilled artisan would clearly comprehend the invention to be practiced in a eukaryotic cell. Indeed, while the proteasome is also present in numerous prokaryotic organisms, it is understood that ubiquitin, and the ubiquitin conjugating system, are not (see e.g. Glickman (2000) Semin Cell Dev Biol Jun; 11(3):149-58).

The Office Action still further states that "the specification does not teach the method as applied for degradation of a polypeptide in a live organism". Applicants submit that there is ample evidence that *in vivo* "live organism" embodiments of the claimed invention are enabled by the instant application and credible to a person of skill in the art at the time the invention was made. This is illustrated, for example, by mere observation of the biotechnology industry. The progress in clinical trials, the development of further gene therapy techniques, and the creation of numerous gene therapy companies and gene therapy divisions of large pharmaceutical companies strongly suggests that "live organism" *in vivo* methods are more than merely a promise. Indeed, a review of cancer gene therapy (see e.g. Roth et al. (1997) J. Natl. Cancer Inst., 89: 21-39) demonstrates in considerable detail the clinical trials in progress and the results achieved thereby in "live organism" applications. The art is replete with examples wherein gene therapy protocols have been used effectively *in vivo* using gene therapy methodologies analogous to those taught by Applicants. The Examiner's attention is directed to, for example, Yang et al. ((1995) Nature Medicine 1: 1052-1056), which teaches "live organism" methods of gene therapy to treat cancer. Yang et al. demonstrates successful transfection of up to 95% of Renca tumor cells in mice using a vector bearing a p21 gene, thereby causing tumor growth inhibition and tumor shrinkage. Also, Jin et al. ((1995) Cancer Research 55: 3250-3253) successfully inhibited tumor growth in mice by transfecting cells with a $p16^{INK4}$ gene (p. 3252-3253). Furthermore, the advanced state of the art of "live organism" *in vivo* gene delivery is by no means limited to that applicable to the treatment of cancer. Indeed, a survey of the literature indicates that methods of promoting tissue healing using "live organism" *in vivo* methods were known to the skilled artisan at the time of the invention. For example, the healing of injured ligament in rats has been shown to be promoted by direct administration of a platelet-derived growth factor cDNA, demonstrating that gene transfer techniques may be used to promote healing of soft tissue wounds (see e.g. Nakamura et al. (1998) Gene Ther 5: 1165-70).

Furthermore, methods for the facile delivery of localized transient gene therapy for the augmentation of fracture healing are known in the art (see e.g. Goldstein & Bonadio (1998) Clin Orthodp 355Suppl: S154-62; and Niyibizi et al. (1998) Clin Orthodp 355Suppl: S148-53). Thus, Applicants' arguments submitted to date have established success in related fields of "live organism" *in vivo* methods. Furthermore, the Office Action provides no evidence which refutes the teachings provided by Applicants in the specification. Accordingly, Applicants submit that the specification in conjunction with the contemporary knowledge in the art supports the claimed *in vivo* methods and constructs in "live organisms" and allows the skilled artisan to practice the claimed invention without undue experimentation.

In conclusion, for the reasons stated above, reconsideration and withdrawal of the rejection of claims 1-11, 14 and 15 as lacking enablement under 35 U.S.C. 1st paragraph is respectfully requested.

Rejections Under 35 U.S.C. §112, second paragraph-Indefiniteness

The Office Action states that claims 1, 5 and 11 have been rejected under 35 US U.S.C. § 112, second paragraph as being "indefinite for failing to particularly point out and distinctly claims the subject matter which applicant regards as the invention." In particular, the Office Action states that the phrase "said ubiquitin protein ligase polypeptide-target polypeptide interaction domain hybrid" lacks sufficient antecedent basis in the claims. Accordingly, claim 1 has been amended to supply the requested antecedent basis for this limitation in the claim.

The Office Action further states that the term "an SCF polypeptide" in claim 5 "is confusing because SCF is a complex, no component of which has the ubiquitin protein ligase activity," and that this language is confusing "(f)or the same reason (that) claim 11 is unclear". Applicants note that the term "an SCF polypeptide" is meant to refer to any of the components of this ubiquitin protein ligase complex and is specifically defined in the application as such (see, e.g. the application at page 9, lines 4-5). Moreover, Applicants repeat their above-made traversal of the rejection of claim 11 for lack of enablement. In particular, the invention is not limited to the use of any particular component of the SCF complex to generate the "ubiquitin protein ligase polypeptide-target polypeptide interaction domain hybrid" fusion of the invention. Accordingly,

and further in light of the fact that the law allows the applicant for patent to be his own lexicographer, Applicants respectfully request reconsideration and withdrawal of this rejection.

CONCLUSION

For the foregoing reasons, Applicants respectfully request reconsideration and withdrawal of the pending rejections. Applicants believe that the claims now pending are in condition for allowance, and notification of such is respectfully requested. If for any reason a telephonic conference with the Applicant would be helpful in expediting prosecution of the instant application, the Examiner is invited to call Applicants' Agent at (617) 832-1764.

If there are any other fees due in connection with the filing of this Response, please charge the fees to our Deposit Account No. 06-1448.

Respectfully submitted,
FOLEY, HOAG, & ELIOT

December 6, 2001

James T. Olesen
Reg. No. 46,967
Agent for Applicants

Patent Group
Foley, Hoag & Eliot LLP
One Post Office Square
Boston, MA 02109-2170
Telephone: (617) 832-1000
Facsimile: (617) 832-7000

VERSION WITH MARKINGS TO SHOW CHANGES MADE

AMENDMENTS TO THE SPECIFICATION

The paragraph beginning at page 10, line 19, has been amended as follows:

The invention also provides methods of treating a cell to stabilize a target polypeptide of ubiquitin protein ligase by contacting the cell with a preparation containing an effective amount of an organic compound which can competively inhibit interaction of the target polypeptide with the ubiquitin protein ligase. In preferred embodiments, the organic compound is a peptide or peptidomimetic, preferably one which is a competitive inhibitor of a WD domain, and most preferably one which includes the general chemical strucutre specified by the formula: G-H-X (3-6)-h-X-X-h-X-r-X-t (2-3)-p-X-h-h-X-X-X-X-D-X-X-X-X-h-W-D (SEQ ID No. 14).

The paragraph beginning at page 40, line 27 and continuing through page 41, line 15, has been amended as follows:

The molecular marker of the test polypeptide is meant to facilitate identification of a target polypeptide interaction domain and isolation of its encoding nucleic acid. In the yeast two-hybrid embodiment of the target polypeptide trap invention, the molecular marker is typically a transcriptional activation domain which functions in yeast and which is a component of the second hybrid gene. It is understood that the second hybrid gene of the present invention can encode any of a number of alternative transcriptional activation domains including the GAL4 transcriptional activation domain region II, the strong transcriptional activator VP16, the weak synthetic transcriptional activators B17 and B112, or the amphipathic helix domain described in Giniger and Ptashne ((1987) Nature 330:670). Modifications of the transcriptional activation can be particularly useful when attempting to either increase or decrease the sensitivity of the target polypeptide trap screen. In the method of the present invention the second hybrid gene may further contain, in addition to a transcriptional activation domain, an optional nuclear localization sequence, such as that of the SV40 Large T antigen encoded by the amino acid sequence PPKKKRKVA (SEQ ID No. 13), which allows for the requisite partitioning of the product of the second hybrid gene in cases where the prey moiety is normally exclusively cytoplasmic. The second hybrid gene may additionally contain an epitope tag, such as hemagluttinin or FLAG, so that production of full length second hybrid gene prey products can be confirmed in a Western

blot. Furthermore, as explained below, the prey epitope tag provides a convenient means of testing for covalent linkage of the bait and prey moieties as is anticipated in some applications of the method of this invention. This determination is conveniently made by means of a Western blot analysis and provides a biochemical means of classifying the clones obtained from a target polypeptide trap screen.

The paragraph beginning at page 59, line 5, has been amended as follows:

In certain embodiments, the inhibitor has a molecular weight of less than 10,000 atomic mass units (amu), more preferably less than 7500 amu, 5000 amu, and even more preferably less than 3000 amu. For instance, the ubiquitin ligase/target polypeptide inhibitor can be either a peptide or peptidomimetic, preferably corresponding in length to a 3-25 mer, e.g., and in certain preferred embodiments, containing a core sequence corresponding to a WD repeat conserved sequence of G-H-X $^{(3-6)}$-h-X-X-h-X-r-X-t $^{(2-3)}$-p-X-h-h-X-X-X-X-D-X-X-X-X-h-W-D (SEQ ID No. 14); wherein "X" indicates any amino acid residue, the number ranges indicated in superscript indicate a variable number of the indicated residue type at that position, "h" indicates a hydrophobic residue, "r" indicates an aromatic amino acid residue, "t" indicates an amino acid residue which stabilizes a tight polypeptide backbone turn such as glycine, proline, aspartic acid or asparagine, and p indicates a polar amino acid residue. In other embodiments the WD repeat competitive inhibitor is provided as a gene construct for expressing the WD repeat peptide. The WD repeat peptide, peptidomimetic or gene construct is formulated in the pharmaceutical preparation for delivery to an animal to be treated.

The paragraph beginning at page 60, line 15 and continuing to page 61, line 2, has been amended as follows:

It is understood that the inhibitors of the invention can be competitive or noncompetitive, and can be general to a class of ubiquitin protein ligase or specific to a particular cellular target protein or somewhat broader in specificity to include multiple cellular protein targets. In preferred embodiments, the present invention provides a peptide, or peptidomimetic that inhibits the ubiqitin-dependent degradation of the target polypeptide. The peptide/ peptidomimetic can, in certain preferred embodiments, range in size from 3-25 amino acid residues. In certain embodiments, a WD repeat inhibitor of the present invention includes a WD repeat core structure

having the formula: G-H-X $^{(3-6)}$-h-X-X-h-X-r-X-t $^{(2-3)}$-p-X-h-h-X-X-X-X-D-X-X-X-X-h-W-D (SEQ ID No. 14), wherein:

G represents a glycine residue, or an analog thereof;

H represents a histidine residue, or an analog thereof;

D represents an aspartic acid residue, or an analog thereof;

W represents a tryptophan residue, or an analog thereof;

and "X" indicates any amino acid residue, "h" indicates a hydrophobic residue, "r" indicates an aromatic amino acid residue, "t" indicates an amino acid residue which stabilizes a tight polypeptide backbone turn such as glycine, proline, aspartic acid or asparagine, and p indicates a polar amino acid residue. While the invention includes all of the groups of inhibitors set forth above, the following descriptions are illustrative of an exemplary WD repeat ubiquitin protein ligase competitive inhibitor of the invention. It is understood that chemical design and other methods described for the WD repeat inhibitor apply broadly to all classes of inhibitor discussed herein.

The paragraph beginning at page 68, line 30 and continuing through page 69, line 4, has been amended as follows:

In a representative embodiment of this method, the amino acid sequences for a population of WD motifs are aligned, preferably to promote the highest homology possible. Amino acids which appear at each position of the aligned sequences are selected to create a degenerate set of combinatorial sequences. To illustrate, multiple WD repeat containing proteins are aligned and, based on these alignments, combinatorial libraries can be generated representing WD repeat peptides which have an amino acid sequence that includes a WD core sequence represented by the formula:

G-H-X $^{(3-6)}$-h-X-X-h-X-r-X-t $^{(2-3)}$-p-X-h-h-X-X-X-X-D-X-X-X-X-h-W-D (SEQ ID No. 14).

The paragraph beginning at page 72, line 27 and continuing through page 73, line 8, has been amended as follows:

While not wishing to be bound by any particular theory, it is noted that hydrophilic polypeptides may be also be physiologically transported across the membrane barriers by

coupling or conjugating the polypeptide to a transportable peptide which is capable of crossing the membrane by receptor-mediated transcytosis. Suitable internalizing peptides of this type can be generated using all or a portion of, e.g., a histone, insulin, transferrin, basic albumin, prolactin and insulin-like growth factor I (IGF-I), insulin-like growth factor II (IGF-II) or other growth factors. For instance, it has been found that an insulin fragment, showing affinity for the insulin receptor on capillary cells, and being less effective than insulin in blood sugar reduction, is capable of transmembrane transport by receptor-mediated transcytosis and can therefor serve as an internalizing peptide for the subject transcellular peptides and peptidomimetics. Preferred growth factor-derived internalizing peptides include EGF (epidermal growth factor)-derived peptides, such as CMHIESLDSYTC (SEQ ID No. 15) and CMYIEALDKYAC (SEQ ID No. 16); TGF- beta (transforming growth factor beta)-derived peptides; peptides derived from PDGF (platelet-derived growth factor) or PDGF-2; peptides derived from IGF-I (insulin-like growth factor) or IGF-II; and FGF (fibroblast growth factor)-derived peptides.

The paragraph beginning at page 73, line 23, has been amended as follows:

A particularly preferred pH-dependent membrane-binding internalizing peptide in this regard is aa1-aa2-aa3-EAALA(EALA)4-EALEALAA-amide (SEQ ID No. 17), which represents a modification of the peptide sequence of Subbarao et al. (Biochemistry 26:2964, 1987). Within this peptide sequence, the first amino acid residue (aa1) is preferably a unique residue, such as cysteine or lysine, that facilitates chemical conjugation of the internalizing peptide to a targeting protein conjugate. Amino acid residues 2-3 may be selected to modulate the affinity of the internalizing peptide for different membranes. For instance, if both residues 2 and 3 are lys or arg, the internalizing peptide will have the capacity to bind to membranes or patches of lipids having a negative surface charge. If residues 2-3 are neutral amino acids, the internalizing peptide will insert into neutral membranes.

The paragraph beginning at page 74, line 31 and continuing through page 75, line 4, has been amended as follows:

An exemplary accessory moiety in this regard would be a peptide substrate for N-myristoyl transferase, such as GNAAAARR (SEQ ID No. 18) (Eubanks et al., in: Peptides. Chemistry and Biology, Garland Marshall (ed.), ESCOM, Leiden, 1988, pp. 566-69) In this

construct, an internalizing peptide would be attached to the C-terminus of the accessory peptide, since the N-terminal glycine is critical for the accessory moiety's activity. This hybrid peptide, upon attachment to a WD peptide or peptidomimetic at its C-terminus, is N-myristylated and further anchored to the target cell membrane, e.g., it serves to increase the local concentration of the peptide at the cell membrane.

The paragraph beginning at page 75, line 23, and continuing through page 76, line 2, has been amended as follows:

In another embodiment of this aspect of the invention, an accessory peptide can be used to enhance interaction of the WD peptide or peptidomimetic with the target cell. Exemplary accessory peptides in this regard include peptides derived from cell adhesion proteins containing the sequence "RGD", or peptides derived from laminin containing the sequence CDPGYIGSRC (SEQ ID No. 19). Extracellular matrix glycoproteins, such as fibronectin and laminin, bind to cell surfaces through receptor-mediated processes. A tripeptide sequence, RGD, has been identified as necessary for binding to cell surface receptors. This sequence is present in fibronectin, vitronectin, C3bi of complement, von-Willebrand factor, EGF receptor, transforming growth factor beta , collagen type I, lambda receptor of E. Coli, fibrinogen and Sindbis coat protein (E. Ruoslahti, Ann. Rev. Biochem. 57:375-413, 1988). Cell surface receptors that recognize RGD sequences have been grouped into a superfamily of related proteins designated "integrins". Binding of "RGD peptides" to cell surface integrins will promote cell-surface retention, and ultimately translocation, of the polypeptide.

The paragraph beginning at page 76, line 13 and continuing to page 77, line 15, has been amended as follows:

In an exemplary embodiment, a WD peptide or peptidomimietic is engineered to include an integrin-binding RGD peptide/SV40 nuclear localization signal (see, for example Hart SL et al., 1994; J. Biol. Chem.,269:12468-12474), such as encoded by the nucleotide sequence provided in the Nde1-EcoR1 fragment:
catatgggtggctgccgtggcgatatgttcggttgcggtgctcctccaaaaaagaagagaaag-gtagctggattc (SEQ ID No. 20), which encodes the RGD/SV40 nucleotide sequence:
MGGCRGDMFGCGAPP-KKKRKVAGF (SEQ ID No. 21). In another embodiment, the

protein can be engineered with the HIV-1 tat(1-72) polypeptide, e.g., as provided by the Nde1-EcoR1

fragment:catatggagccagtagatcctagactagagccc-tggaagcatccaggaagtcagcctaaaactgcttgtaccaattgctattg taaaaagtgttgctttcattgccaagtttgtttcataacaaaagcccttggcatctcctatggcaggaagaagcggagacagcgacgaagacc tcctcaaggcagtcagactcatcaagtttctctaagtaagcaaggattc (SEQ ID No. 22), which encodes the HIV-1 tat(1-72) peptide sequence:

MEPVDPRLEPWKHPGSQPKT-ACTNCYCKKCCFHCQVCFITKALGISYGRKKRRQRRRP PQGSQTHQVSLSKQ (SEQ ID No. 23). In still another embodiment, the fusion protein includes the HSV-1 VP22 polypeptide (Elliott G., O'Hare P (1997) Cell, 88:223-233) provided by the Nde1-EcoR1 fragment:

cat atg acc tct cgc cgc tcc gtg aag tcg ggt ccg cgg gag gtt ccg cgc gat gag tac gag gat ctg tac tac acc ccg tct tca ggt atg gcg agt ccc gat agt ccg cct gac acc tcc cgc cgt ggc gcc cta cag aca cgc tcg cgc cag agg ggc gag gtc cgt ttc gtc cag tac gac gag tcg gat tat gcc ctc tac ggg ggc tcg tca tcc gaa gac gac gaa cac ccg gag gtc ccc cgg acg cgg cgt ccc gtt tcc ggg gcg gtt ttg tcc ggc ccg ggg cct gcg cgg gcg cct ccg cca ccc gct ggg tcc gga ggg gcc gga cgc aca ccc acc acc gcc ccc cgg gcc ccc cga acc cag cgg gtg gcg act aag gcc ccc gcg gcc ccg gcg gcg gag acc acc cgc ggc agg aaa tcg gcc cag cca gaa tcc gcc gca ctc cca gac gcc ccc gcg tcg acg gcg cca acc cga tcc aag aca ccc gcg cag ggg ctg gcc aga aag ctg cac ttt agc acc gcc ccc cca aac ccc gac gcg cca tgg acc ccc cgg gtg gcc ggc ttt aac aag cgc gtc ttc tgc gcc gcg gtc ggg cgc ctg gcg gcc atg cat gcc cgg atg gcg gcg gtc cag ctc tgg gac atg tcg cgt ccg cgc aca gac gaa gac ctc aac gaa ctc ctt ggc atc acc acc atc cgc gtg acg gtc tgc gag ggc aaa aac ctg ctt cag cgc gcc aac gag ttg gtg aat cca gac gtg gtg cag gac gtc gac gcg gcc acg gcg act cga ggg cgt tct gcg gcg tcg cgc ccc acc gag cga cct cga gcc cca gcc cgc tcc gct tct cgc ccc aga cgg ccc gtc gag gaa ttc (SEQ ID No. 24)

which encodes the HSV-1 VP22 peptide having the sequence:

MTSRRSVKSGPREVPRDEYEDLYYTPSSGMASPDSPPDTSRRGALQTRSRQRGEVRFVQ YDESDYALYGGSSSEDDEHPEVPRTRRPVSGAVLSGPGPARAPPPPAGSGGAGRTPTTAP RAPRTGRVATKAPAAPAAETTRGRKSAQPESAALPDAPASTAPTRSKTPAQGLARKLHF STAPPNPDAPWTPRVAGFNKRVFCAAVGRLAAMHARMAAVQLWDMSRPRTDEDLNE LLGITTIRVTVCEGKNLLQRANELVNPDVVQDVDAATATRGRSAASRPTERPRAPARSA SRPRRPVE (SEQ ID No. 25).

The paragraph beginning at page 77, line 16, has been amended as follows:

In still another embodiment, the fusion protein includes the C-terminal domain of the VP22 protein from, e.g., the nucleotide sequence (Nde1-EcoR1 fragment):
cat atg gac gtc gac gcg gcc acg gcg act cga ggg cgt tct gcg gcg tcg cgc ccc acc gag cga cct cga gcc cca gcc cgc tcc gct tct cgc ccc aga cgg ccc gtc gag gaa ttc (SEQ ID No. 26)
which encodes the VP22 (C-terminal domain) peptide sequence:
MDVDAATATRGRSA-ASRPTERPRAPARSASRPRRPVE (SEQ ID No. 27)
In certain instances, it may also be desirable to include a nuclear localization signal as part of the WD peptide.
In the generation of fusion polypeptides including the subject WD peptides, it may be necessary to include unstructured linkers in order to ensure proper folding of the various peptide domains, and prevent steric or other inteference of the heterologous domains with the PV inhibitory activity of the WD peptide. Many synthetic and natural linkers are known in the art and can be adapted for use in the present invention, including the (Gly3Ser)4 (SEQ ID No. 28) linker.

The paragraph beginning at page 80, line 17, has been amended as follows:

The pseudodipeptide is then coupled at the C-terminus, according to the above example, with a suitably protected tyrosine residue, and at the N-terminus with a protected alanine residue, by standard techniques, to yield the protected tetrapeptide isostere A-I-Y-Y (SEQ ID No. 29). The terapeptide is then further condensed with the olefinic tripeptide analog derived by similar means for Lys-Ala-Arg, and so forth to build up the full WD peptide. The protecting groups are then removed with strong acid to yield the desired peptide analog, which can be further purified by HPLC.

The paragraph beginning at page 142, line 29 and continuing to page 144, line 10, has been amended as follows:

S. cerevisiae INVSc1 (Invitrogen) and Y81 cells (gifts of S. Elledge) were used to assess the *in vivo* degradation of the papillomavirus E2 or pRB by the engineered Cdc4p ubiquitin-protein ligases. The human osteosarcoma Saos-2 cell was a gift of Drs. David Thomas and Philip Hinds. The galactose inducible pRB expression plasmid p2202TRB was a gift of Dr. Robert Weinberg (Hatakeyama et al. (1994) Genes Dev. 8: 1759-71). The engineered Cdc4p

derivatives designed for targeting pRB degradation were constructed as follows: Cdc4p$^{F/WD}$-LTP and Cdc4p$^{F/WD}$-LTP(FGSK) cDNAs were generated by PCR using the 5' primer 5'-GCGGATCCACCATGGATAAMAAAGAGGGACCTAATAAC-3' (SEQ ID No. 30) that hybridizes to *CDC4* corresponding to residues 270 to 277 with a BamHI site and the ATG translation initiation codon at the 5' end, and the 3' primer encoding sequences of either the Cdc4p C-terminus (residues -779) in frame fused to the pRB binding domain of the SV40 large T antigen (residues 103-115), or the same primer carrying point mutations of the LFCSE (SEQ ID No. 47) motif that abolishes its interaction with pRB (DeCaprio et al. (1989) Cell 58:1085-95; Dyson et al. (1989) Science 243: 934-7; Figge et al. (1993) Protein Sci 2: 155-64; Munger et al. (1989) EMBO J 8: 4099-105); Yang et al. (1995) Nucleic Acids Res 23: 1152-6). The sequences of these two 3' primers carrying the NotI site and a translation stop codon are 5'-GCGCGGCCGCCTACTCATCATCACTAGATGGCAMCTTCTGAGCAAAACAG CCCTCTGG TATTATAGTTGTCCTCGT-3' (SEQ ID No. 31) and 5'-CGCGGCCGCCTACTCATCATCACTAG ATGGCAMTTGAGCCAAAGTTTTCTCTGGTATTATAGTTGTCCTCGT-3' (SEQ ID No. 32). The resulting PCR fragments encoding Cdc4p$^{F/WD}$-LTP or Cdc4p$^{F/WD}$-LTP(FGSK) hybrids were digested with BamHI and NotI, and were subsequently cloned into the pYES2 vector (Invitrogen) for expression in Y81 cells under the control of the *GAL1,10* promoter. The Cdc4p$^{F/WD}$-E7N or Cdc4p$^{F/WD}$-E7N(DLYC) hybrid constructs were obtained by a two-step PCR approach as described in detail (Dieffenbach, 1995). The first PCR reactions for making individual Cdc4p$^{F/WD}$-E7N or E7N(DLYC) DNA fragments were conducted using pYES-F-CDC4 (this work), pGST-E7(2-35) or pGST-E7(2-35)(DLYC) plasmids as templates (Gifts of Dr. K. Munger). The sequences for the primer sets used are as follows: 5'-GCWATCCACCATGGATAATITAAAGAGGGACCTAATAAC-3' (SEQ ID No. 33) (5'-extreme) and 5'-GTAGGTGTATCTCCATGTGGTATrATAGTrGTCC-3' (SEQ ID No. 34) for Cdc4p$^{F/WD}$, 5'-GGACAACTATAATACCACATGGAGATACACCTAC-3' (SEQ ID No. 35) and 5'-GCCTCGAGTCACTCCTCCTCTGAGCTGTC-3' (SEQ ID No. 36) (3'-extreme) for E7N or E7N(DLYC), respectively. The second step PCR reactions were conducted using the same 5'- and 3' extreme primers to ligate Cdc4p$^{F/WD}$/E7N or Cdc4p$^{F/WD}$/E7N(DLYC) PCR fragments together. The resulting hybrid Cdc4p$^{F/WD}$-E7N or Cdc4p$^{F/WD}$-E7N(DLYC) DNA fragments

were digested with BamHI and XhoI which were introduced by the 5'- and 3'- extreme primers, respectively, and were cloned into the p426-ADH plasmid (ATCC) for constitutive expression in Y81 cells under the control of the ADH promoter. Cdc4p$^{F/WD}$-EIC were constructed similarly by the two-step PCR approach using primer pairs 5'-GCGGATCCACCATGGATAAMAAAGAGGGAC CTAATAAC-3' (SEQ ID No. 37) (5'-extreme)-3' and 5'-CCTATCACATCTATATTTTATTGGTATTA TAGTTGTC-3' (SEQ ID No. 38) for Cdc4p$^{F/WD}$, and 5'- GACAACTATAATACCAATAAAATATAG ATGTGATAGG-3' (SEQ ID No. 39) and 5'-GCCTCGAGTCATAATGTGTTAGTATTTTGTCCTG-3' (SEQ ID No. 40) for E1C. The resulting Cdc4p$^{F/WD}$-EIC fragment was cloned into the BamHI/XhoI sites of p426-ADH vector. EE-tagged HPV16-E2 and HPV16-E2(E39A) were generated by PCR using primers 5'-GCGGATCCACCATGGAGGAAGAAGAGTATATGCCCA TGGAGGAGACTCTT TGCCAACGTTTTAAATGTG-3' (SEQ ID No. 41) and 5'-GCGCGGCCGCTCATATAGACATAAATCCA GTAGAC -3' (SEQ ID No. 42), and the resulting PCR fragments were cloned into the single copy plasmid pCM185 for expression under the control of the tetracycline-repressible (tetO^{R}) promoter in *S. cerevisiae*.

The paragraph beginning at page 144, line 11, has been amended as follows:

βTrCp-E7N and βTrCP-E7N(DLYC) were also constructed by the two step PCR approach with primer pairs 5'-GCGGATCCGCCACCATGGACTACAAGGACGAC GATGACAAAGATGACCCGGCCGAGGCGGTGCTG-3' (SEQ ID No. 43) and 5'-GTAGGTGT ATCTCCATGTCTGGAGATGTAGGTGTATG-3' (SEQ ID No. 44) for PTRCP, 5'-CATACACCTA CATCTCCAGACATGGAGATACACCTAC-3' (SEQ ID No. 45) and 5'-GCGCGGCCGCTCACTCCTCCTCTGAGCTGTC-3' (SEQ ID No. 46) primer sets for E7N or E7N(DLYC). The final PCR fragments were cloned into the BamHI/NotI sites of pcDNA3 (Invitrogen). The cloned PCR fragments described above were sequenced by the BCMP core facility at Harvard Medical School. Expression of the engineered Cdc4p hybrids in Y81 cells were confirmed by immunoblotting using the anti-Cdc4p polyclonal antibody.

AMENDMENTS TO THE CLAIMS

1. **(Amended)** A method for targeting degradation of a polypeptide in vivo comprising:

providing a ubiquitin protein ligase polypeptide-target polypeptide interaction domain hybrid comprising a ubiquitin protein ligase polypeptide that encodes a ubiquitin conjugation activity, which is functionally linked to a target polypeptide interaction domain that provides a target polypeptide recruitment activity, wherein the ubiquitin protein ligase polypeptide is an SCF polypeptide, a HECT polypeptide, or a UBR1 polypeptide;

expressing said ubiquitin protein ligase polypeptide-target polypeptide interaction domain hybrid in a cell;

recruiting the target polypeptide to said ubiquitin protein ligase polypeptide; and

ubiquitinating the target polypeptide,

thereby forming a ubiquitin-target polypeptide conjugate which is targeted for degradation.

5. **(Canceled)** The method of claim 4, wherein the E3 ubiquitin protein ligase is selected from the group consisting of: an SCF polypeptide, a HECT polypeptide, and a UBR1 polypeptide.

6. **(Amended)** The method of claim [5] 1, wherein the SCF polypeptide is an F-box polypeptide.

11. **(Amended)** The method of claim [5] 1, wherein the ubiquitin protein ligase polypeptide is an SCF polypeptide [is] selected from the group consisting of: a Cdc4 polypeptide, a Skp1 polypeptide or a cullin polypeptide.